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An FcγRII-, FcγRIII-Specific Monoclonal Antibody (2.4G2) Decreases Acute *Trypanosoma cruzi* Infection in Mice

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In order to study the role of FcγRs in *Trypanosoma cruzi* infection in mice, the 2.4G2 monoclonal antibody (MAb), specific to the extracellular domains of FcγRII and FcγRIII, was injected intraperitoneally into mice. Flow cytometry studies of uninfected mice showed that 2.4G2 MAb bound to peritoneal and lymph node cells, respectively, on days 2 and 6 after injection. Repeating 2.4G2 injections every 3 to 4 days decreased the availability of FcγRs on peritoneal, lymph node, and spleen cells. Injections of 2.4G2 MAb into *T. cruzi*-infected mice, at days −1, 3, 7, 11, 16, 20, and 24 relative to infection, reduced mortality in comparison with that in infected animals injected with an unrelated MAb (50 versus 93.3% mortality; $P < 0.01$). Parasitemia in 2.4G2-treated mice was significantly (three times) lower than in control animals on days 21 and 24 postinfection ($P < 0.05$), before parasite-specific antibodies were detectable at significant levels. Immunoglobulin and *T. cruzi*-specific antibody levels were similar in all groups of mice. These results indicate that repeated injections of 2.4G2 MAb administered to acutely infected mice reduce the in vivo infection level, suggesting that FcγRs play a role in the early host invasion by *T. cruzi* parasites.

Chagas' disease is caused by the protozoan flagellate *Trypanosoma cruzi*. In a previous work, we showed that *T. cruzi* infection in mice, the murine model of human Chagas' disease, induced an upregulation of the expression of low-affinity FcγRII and FcγRIII at the cell surface and an increase in the number of FcγRII-, FcγRIII-positive [FcγRII/III(+)] cells. This effect occurred before as well as after the rise in *T. cruzi*-specific antibody response (2). Low-affinity FcγRs are expressed on lymphoid and myeloid lineages (11, 14). They bind complexed immunoglobulin G1 (IgG1), IgG2a, and IgG2b antibodies, which have been shown to be involved in the in vivo clearance of circulating *T. cruzi* parasites (5). Indeed, besides their complement-mediated lytic capacity, antibodies contribute to parasite elimination by cell cytotoxicity and phagocytosis (4). If an increased availability of FcγRs borne by effector cells can therefore contribute to the host protection when parasite-specific antibodies are available, the significance of FcγR upregulation in the early infection, before antibodies are detectable, is not clear. In order to investigate the role of FcγRs in the course of *T. cruzi* in vivo infection, mice were injected with 2.4G2, a FcγRII/III-specific monoclonal antibody (MAb), before and after *T. cruzi* infection. 2.4G2 binds specifically and with high affinity the extracellular domains of FcγRII and FcγRIII, and it has been previously shown to block the in vivo clearance of immune complexes (15). In the present work, we show that repeated injections of 2.4G2 MAb reduce both mortality and parasitemia of infected mice and decrease the availability of the FcγRs on cell membranes.

The 2.4G2 MAb (a rat IgG2b) and an unrelated control MAb of the same rat isotype (IR863) were used for intraperitoneal (i.p.) injections in mice (8-week-old female BALB/cJ). The MAbs were prepared by precipitation with 18% Na_2SO_4 from ascitic fluids. Mouse infection was performed by one i.p. injection of blood from acutely infected mice containing 100 *T. cruzi* trypomastigotes (Tehuantepec strain), as previously described (8). Control animals received blood from uninfected mice. Mortality was recorded daily, and parasitemia was determined by testing tail blood (8).

2.4G2 MAb was searched for in plasma by indirect immunofluorescence (IF) on P815 cells [a FcγRII/III(+) cell line] and by inhibition of rosette formation between P815 cells and sheep erythrocytes coated with mouse monoclonal IgG2a, as previously described (12). The limit of detection of 2.4G2 was around 10 to 100 ng/ml. Flow cytometry analyses were performed to study the effect of 2.4G2 or IR863 MAbs on peritoneal cells (PC), mesenteric lymph node cells (LNC), and/or spleen cells (SC). Cells from two to four mice from each group were pooled, washed twice in balanced salt solution (BSS) supplemented with 5% fetal calf serum, and adjusted to the required concentration of trypan blue-excluding cells. All cell processings were performed in an ice bath. Direct IF was used to assess in vivo cell-bound 2.4G2 or IR863 MAbs. Samples of 5×10^5 cells were incubated for 30 min in BSS or with $F(ab')_2$ fragments of fluorescein isothiocyanate (FITC)-labelled mouse anti-rat immunoglobulins (10 μg/ml) (FITC-MAR) (Jackson Immuno Research Laboratory, West Grove, Pa.). Indirect IF was used to assess total FcγRs (free and occupied). Cell samples were incubated with BSS or with 10 μg of 2.4G2 $F(ab')_2$ fragments per ml (12) for 30 min at 0°C, washed three times in BSS-fetal calf serum, and incubated with FITC-MAR for 30 min at 0°C. Finally, cells were washed, fixed in 0.5% paraformaldehyde, and diluted in phosphate-buffered saline (PBS)–1% bovine serum albumin–0.2 M NaN_3, and then fluorescence was analyzed in a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.) after gating to eliminate dead cells and fragments. The levels of *T. cruzi*-specific antibodies and

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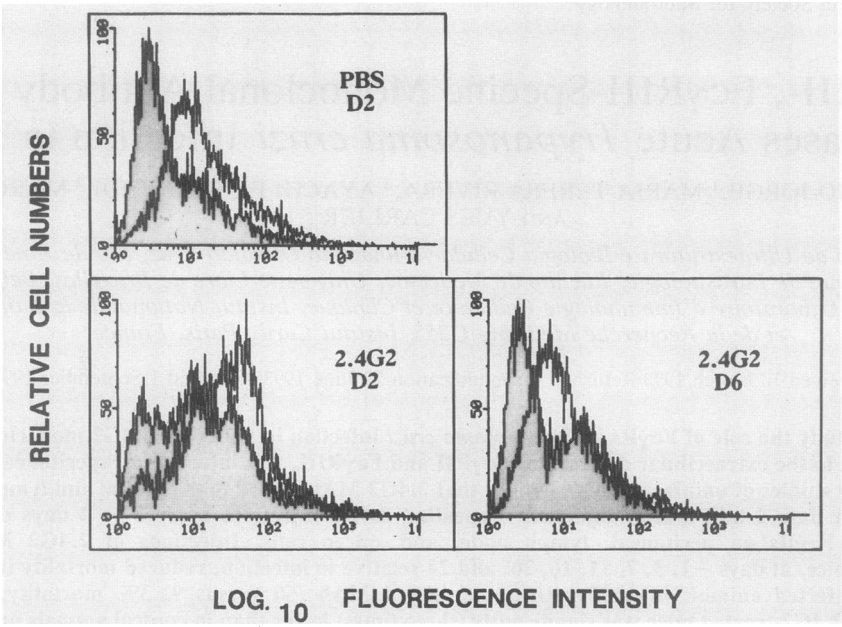

FIG. 1. Results of IF study (flow cytometry) of PC from uninfected mice having received a single i.p. injection of PBS or of 2.4G2 MAb. Shaded areas correspond to the binding of FITC-MAR $F(ab')_2$ fragments on the cell surface and indicate the in vivo binding of 2.4G2 on cells (direct IF). Open areas correspond to the binding of $F(ab')_2$ fragments of 2.4G2 prior to the binding of FITC-MAR $F(ab')_2$ fragments and assess the expression of FcγRII/III on cells (indirect IF). Cells were collected 2 (D2) or 6 (D6) days after injection.

of total immunoglobulins of isotypes G1, G2a, and G2b were measured in mouse plasma by enzyme-linked immunosorbent assay, as previously described (6).

Effect of 2.4G2 in noninfected mice. In order to study the fate of injected MAb in blood and on cells, three groups of uninfected mice were inoculated by one i.p. injection (200 μl) of either PBS or 2.4G2 or IR863 MAb, adjusted to 8 μg of protein per g of body weight, as used in a previous in vivo study (15). No 2.4G2 activity was detected by IF or rosette inhibition in plasma 2, 4, or 6 days after injection. In contrast, 2.4G2 was found on cells. Figure 1 shows representative results obtained with PC, by flow cytometry. Direct IF was strongly increased at day 2 and only weakly increased on day 6 in PC from 2.4G2-treated mice, in comparison with that in cells from PBS- and IR863-treated mice. Indirect IF of PC increased also at day 2 but recovered to a normal value on day 6 after the injection of 2.4G2. Results obtained on day 4 were intermediate between those obtained on days 2 and 6. IR863 increased slightly indirect IF on PC at day 2. Comparison of IF data for LNC from 2.4G2-treated mice with those for control animals indicated that 2.4G2 was detectable at day 6 (direct IF), without modification of the FcγR expression on the cell surface (indirect IF).

On the basis of the above-described results, indicating the persistence of 2.4G2 on cells for at least 4 to 6 days after a single injection, the effect of prolonged exposure to 2.4G2 in vivo was studied. Four uninfected mice per group received six injections of PBS, 2.4G2, or IR863 every 3 to 4 days. The first injection was adjusted to 8 μg of protein per g, whereas the subsequent ones were with 5 μg of protein per g. Flow cytometry analysis of cells collected 2 days after the last injection indicated that repeated injections of 2.4G2 decreased both the number of LNC and SC expressing FcγRs on their membrane and the number of FcγRs on PC (indirect IF). 2.4G2 and IR863 were bound on PC but not on SC and LNC (direct IF). A reduced availability of FcγRs was found 2 days after the last injection but not after 7 days. This protocol therefore decreased the number of available FcγRII/IIIs in vivo for a total period of 3 weeks.

Effect of 2.4G2 in *T. cruzi*-infected mice. PBS or 2.4G2 or IR863 MAbs were injected into *T. cruzi*-infected mice (14 to 16 per group) on days −1, 3, 7, 11, 16, 20, and 24 relative to infection. Cumulative mortality during the course of infection in the 2.4G2 and IR863 groups of mice is shown in Fig. 2. At the end of the experiment (day 36 p.i.), only 7 of the 14 (50%) 2.4G2-treated mice had died, whereas mortality reached 14 of 15 (93.3%) in the IR863-treated mice (X^2 = 10.2; $P < 0.01$). The kinetics of mean parasitemia, studied every 3 to 4 days in both groups of mice, are shown in Fig. 2. Parasites were detected at the same time in both groups, on the 14th day p.i., and at similar levels until day 17 p.i. On days 21 and 24 p.i., when the mean parasitemia of the control group (IR863 treated) peaked, the mean parasite level of the 2.4G2-injected group remained about three times lower ($P < 0.05$ by the Mann-Whitney-Wilcoxon test). It began to decrease, as in the IR863 controls, on day 28 p.i., i.e., 4 days after the last injection. No difference in parasitic parameters between mice injected with IR863 and those injected with PBS could be observed.

Parallel variations in kinetics of both immunoglobulins and *T. cruzi*-specific antibodies of IgG1, IgG2a, and IgG2b isotypes were seen in the three groups of mice. *T. cruzi* infection increased the plasma concentrations of IgG2a and, to a lesser extent, of IgG1 and IgG2b. Both IgG2 isotypes increased from the third week p.i., while IgG1 increased later, on the fourth week p.i. *T. cruzi*-specific antibodies were detected in plasma after the third week p.i., reaching significant levels from the fourth week p.i., i.e., during the clearance of blood parasites.

Our study shows that repeated injections of 2.4G2 modified the course of *T. cruzi* acute infection in mice and

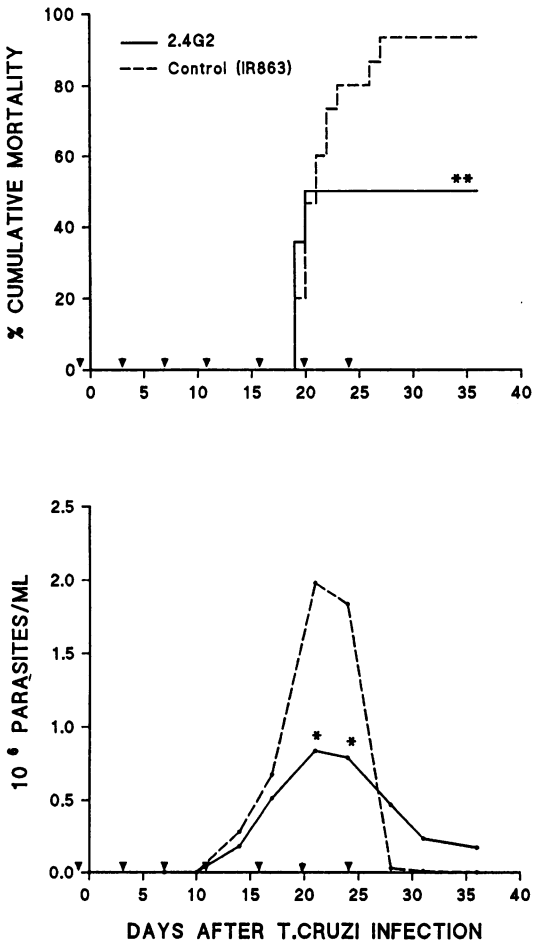

FIG. 2. Cumulative mortality and parasitemia curves during the course of *T. cruzi* infection in mice treated with 2.4G2 or IR863 MAbs. Results shown are the geometric means of individual values (14 to 16 mice per group). Arrows indicate time points of i.p. injection of MAbs, and asterisks indicate time points at which differences between 2.4G2-treated and control mouse groups were statistically significant (*, $P < 0.05$; **, $P < 0.01$).

reduced mortality and parasitemia of animals, before significant levels of *T. cruzi*-specific antibodies became detectable. 2.4G2 injections did not modify the humoral immune response of mice but decreased the FcγRII/III availability on cell membranes. No effect was found in animals injected with an unrelated MAb of the same isotype or with PBS.

The fate of 2.4G2 in vivo was studied in blood and on cell membranes. Its absence in blood collected 2 or more days after injection is consistent with the results of a previous study by Kurlander et al. (15). In addition, our study shows the binding of 2.4G2 to cellular FcγRs in vivo. Use of the i.p. route explains the binding to PC, observed by direct IF after 2 days, when other organs, such as lymph nodes, were not reached earlier than 6 days after injection. 2.4G2 not only bound to a large proportion of cells bearing FcγRII/III; it also modulated the expression of membrane FcγRs in vivo. Indeed, 2 days after a single 2.4G2 injection, an increased expression of FcγRs was observed by indirect IF in PC. A similar but weaker effect was observed with the IR863 MAb. This in vivo upregulation of FcγRs by the high-affinity binding of 2.4G2 MAb to FcγRII/III or, to a lesser extent, by the low-affinity binding of the Fc portion of IgG, confirms results previously obtained in vitro (11). When 2.4G2 injections were repeated every 3 to 4 days, however, other effects were observed. Indirect IF indicated that 2.4G2, but not IR863, downregulated the expression of FcγRs on PC and decreased the number of FcγR(+) SC and LNC from uninfected mice sacrificed after six injections. The prolonged presence of 2.4G2 in vivo therefore induced different effects, which were not observed in vitro. Our study does not allow the analysis of the mechanisms involved in the modulation of FcγR expression, but it highlights the complex effects of 2.4G2 in vivo, which induces an upregulation of receptors followed by a long-term decreased availability of FcγRII/III.

Repeated injections of 2.4G2 into *T. cruzi*-infected mice also markedly reduced parasitemia and mortality. The reduced mortality of 2.4G2-treated mice was probably related to a reduction of their parasitic load, as generally observed with our model of *T. cruzi* infection in BALB/c mice (7, 22). This raises the question of the mechanisms by which 2.4G2, injected before and during parasite multiplication in mice, reduces the level of *T. cruzi* infection. Parasite-specific antibodies cannot be involved in such an effect. Indeed, 2.4G2 treatment of mice did not modify the humoral immune response, and the effect on infection was observed when antibodies were still hardly detectable. On the other hand, interactions between FcγRs on host cells and parasites cannot be excluded. FcRs have been considered as adhesion molecules involved in cell-cell interactions (9), and *T. cruzi* is able to invade many cell types, including FcγR-bearing cells, such as macrophages and endothelial cells (17). Moreover, previous reports indicate that *T. cruzi*, like other parasites such as *Trypanosoma musculi* (23), *Leishmania* spp. (13), or *Schistosoma mansoni* (20, 21), expresses FcRs (13, 18), which can enhance its infective capacity (18). Cell-parasite adhesion could also be favored by indirect interactions between host cell FcRs and parasite FcRs, bridged by soluble immune complexes. These are found at high levels during *T. cruzi* infection (6, 19), and the blocked clearance of immune complexes by 2.4G2 in vivo (15) might still enhance their levels. Not only could FcR-dependent interactions enhance adhesion of parasites to host cells, but they could also enhance cell invasion and, therefore, the subsequent intracellular multiplication of parasites. Indeed, the binding to host cell FcγRs triggers the rapid internalization of the ligand-receptor complex (14). The long-term reduction of FcγR availability on murine cells, induced in vivo by 2.4G2 injections, could therefore directly or indirectly reduce infection by limiting host-parasite interactions. Another nonexclusive possible effect of 2.4G2 could be the activation of FcγRIII(+) cells. Indeed, when cross-linked by a divalent ligand such as 2.4G2, FcγRIIIs transduce activation signals, resulting in the release of mediators and cytokines such as gamma interferon and tumor necrosis factor alpha (1, 10, 16). The last two could limit parasite multiplication and enhance the natural cellular mechanisms of infection control occurring before the antibody detection (3, 22).

In conclusion, our observation that repeated injections of 2.4G2 reduce *T. cruzi* infection before the rise of antibody response suggests that FcγRs might play a role in the early regulation of host-parasite relationships. The mechanisms underlying this regulation remain to be identified.

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